REMARKS

The Examiner is thanked for indicating that the application is in sequence compliance.

Formal drawings are attached, and the Brief Description of Drawings is amended to reflect the numbering used in the Figures and to describe each individual panel.

The Office Action requires restriction under 37 CFR §1.499 from among the following:

Group I: claims 1-21, 28 and 33, drawn to a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is CTLA-4;

Group II: Claims 1-11, 13-21, 28 and 33, drawn to a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is CD28;

Group III: Claims 1-11, 13-21, 28 and 33, drawn to a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is ICOS;

Group IV: Claims 22-27, drawn to a polynucleotide encoding a binding moiety, vectors and host cells comprising, and methods of producing the binding moiety by culturing said host cells, wherein the at least one monomeric V-like domain is derived form a non-antibody ligand that is CTLA-4;

Group V: Claims 22-27, drawn to a polynucleotide encoding a binding moiety, vectors and host cells comprising, and methods of producing the binding moiety by culturing said host cells, wherein the at least one monomeric V-like domain is derived form a non-antibody ligand that is CD28;

Group VI: Claims 22-27, drawn to a polynucleotide encoding a binding moiety, vectors and host cells comprising, and methods of producing the binding moiety by culturing said host cells, wherein the at least one monomeric V-like domain is derived form a non-antibody ligand that is ICOS;

Group VII: Claim 29, drawn to a method of treating by administering a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is CTLA-4;

Group VIII: Claim 29, drawn to a method of treating by administering a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is CD-28.

Group IX: Claim 29, drawn to a method of treating by administering a binding moiety wherein the at least one monomeric V-like domain is derived from a non-antibody ligand that is ICOS; and

Group X: Claims 30-32, drawn to a method of selecting a binding moiety.

Additionally, Applicants were required to elect a single species from among those recited in claims 14, 16 and 17.

Applicants elect Group I, and the species somatostatin in claim 14, human antibodies in claim 16 and human anti-melanoma antibody V86 in claim 17, with traverse.

The Office Action alleges that the claims of the present invention are not linked so as to form a single inventive concept under PCT Rule 13.1. Allegedly, the claims of the present invention lack the same or corresponding special technical features as required under PCT Rule 13.2 because the special technical feature of the Group I claims is allegedly present in the prior art.

The special technical feature of the Group I claims is a binding moiety comprising a monomeric V-like domain, wherein at least one CDR loop is modified or replaced such that the solubility of the modified VLD is improved. The Office Action states that Peach *et al.* "teach chimeric molecules in which complementarity determining regions of CD28 and CTLA4 have been exchanged." Office Action at 4.

Contrary to the assertions of the Office Action, the present invention is not described by Peach *et al.* The Office Action acknowledges that Peach *et al.* is silent with respect to the effect of changes in the CDR loop structures on solubility. In other words, Peach *et al.* does not clearly teach the reader to modify CDR loop structures within a monomeric V-like domain in order to increase the solubility of the domain. Indeed, all of the binding domains described in this publication are fused to an Ig constant domain in order to achieve solubility (see page 2050, third full paragraph and page 2051, line 12). In fact, the fusion of the CTLA4 domains to Ig sequences is designed precisely to eliminate the type of aggregation (principally due to hydrophobic interactions of unfused CTLA4 molecules) described in the present specification,

and which the alterations in CDR regions can obviate. In this regard the citation actually teaches away from the present invention.

The objection is based entirely on the Examiner's interpretation of Figure 4. In this experiment, however, all protein applied to the gel has been immunoprecipitated using an antibody against the Fc portion of the molecule, so that any incorrectly folded molecules not recognized by the antibody will be discarded prior to examination. Insoluble aggregate forms of protein, as described in the present application, would therefore not be displayed or detected on this gel.

Peach *et al.* make the following conclusions concerning Figure 4: "Under reducing conditions, the chimeric proteins migrated as species with relative molecular masses between 100 and 150kD, with some proteins having an additional species with relative molecular masses between 50 and 70kD" (page 2052 top right paragraph). Although the authors go on to imply that they observe monomeric and dimeric forms of the fusion proteins on the gel, it is unclear from the relative mobilities of the species in Figure 4 that this is in fact the case. In particular, the molecular weights of these species cannot be readily determined with any precision from the gel as shown. Accordingly, the assumption in the Office Action that the "additional species" represent monomers is not soundly based on the scientific evidence. Notably, Peach *et al.* do not describe these "additional species" as monomeric forms of the protein.

Even if the additional molecular weight species shown in Figure 4 do represent monomeric forms of the fusion proteins, and Applicants maintain that they do not, the citation still fails to teach the claimed invention. The authors mention on page 2052 that the fusion proteins may exist as monomers and disulfide-linked dimers. It is suggested that dimerization of the fusion proteins is due to cysteine 123 in CTLA-4Ig. The separation of disulfide-linked dimers such as these into monomers is not an indication of improved solubility in the sense of the present invention. As explained in the specification at page 7, lines 1 to 8, solubility of the binding domains correlates with the production of correctly folded monomeric domains that do not form aggregates. There is nothing in Peach *et al* to suggest that modification of CDR loops can improve folding and/or reduce aggregation of monomeric V-like domains. Therefore, the present invention is not disclosed or suggested by Peach *et al.*, such that the special technical feature linking the claims is <u>not</u> known in the prior art.

In view of the fact that the Examiner's lack of unity objection is based on erroneous reasoning, all of the Groups of claims should be rejoined in the present application. Consequently, reconsideration and withdrawal of the restriction requirement is respectfully requested.

Turning now to the election of species, the Office Action also requires election of a specific embodiment which replaces the CDR loop structure from among those recited in claims 14, 16 and 17. Allegedly, the species are distinct because each product differs in its structure and physiochemical properties. It is understood that claims 13 and 15 are generic, and that, if they are found to be allowable, all recited species will be considered.

In summary, enforcing the present restriction requirement would result in inefficiencies and unnecessary expenditures by both the Applicants and the PTO, as well as extreme prejudice to Applicants (particularly in view of GATT, a shortened patent term may result in any divisional or continuing applications filed). Restriction has not been shown to be proper, especially since the requisite showings have not been made in the Office Action and there are relationships between all of the pending claims. Indeed, the search and examination of each Group is likely to be co-extensive and, in any event, would involve such interrelated art such that the search and examination of the entire application can and should be made without. All of the foregoing, therefore, mitigate against restriction.

CONCLUSION

Reconsideration and withdrawal of the restriction requirement and election of species, and a favorable examination on the merits is respectfully requested in view of the remarks herein.

Respectfully submitted,

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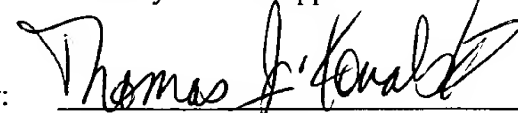
Attorneys for the Applicant

By: 

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

On page 10, line 16 (after "Brief Description of the Drawings"):

--Figures 1A-F show[:] CTLA-4 VLD-Specific Oligonucletides.

Figures 2A and 2B show the[: P]polynucleotide sequence of complete cDNA encoding human CTLA-4 (2A) and polypeptide sequence of the VLD of human CTLA-4 (2B).

Figure 3 shows the phage [: D]display of CTLA-4 VLD STMs as gene 3 fusions on the surface of phage or phagemid. CTLA-4 VLD STMs are depicted as black spheroids; gene 3 protein is depicted as white spheroids; FLAG polypeptide is depicted in grey; genes are marked in a similar colour code and are depicted in an oval phage(mid) vector.

Figure 4 shows a [: S]schematic representation of the somatostatin polypeptide. Somatostatin (somatotropin release-inhibiting factor SRIF) in a cyclic 14-amino acid polypeptide. The cyclic nature is provided by a disulphide linkage between the cysteine residues at positions 3 and 14. The four residues which constitute the tip of the loop (Phe-Trp-Lys-Thr) are implicated in binding to members of the somatostatin receptor family.

Figure 5 shows the [: S]size exclusion HPLC profiles of affinity purified CTLA-4 VLD and CTLA-4-Som3 STM. Recombinant human CTLA-4 proteins were expressed in E. coli host TG1 from vector pGC, purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12 HR column. The elution profiles of purified CTLA-4 VLD and CTLA-4-Soms3 STM are overlayed in this graph. CTLA-4 VLD comprises tetramer (21.86 min), dimer (26.83) and monomer (29.35 min). CTLA-4-Som3 STM comprises dimer (26.34) and monomer (29.28). Traces represent absorbance at 214 nm and are given in arbitrary units.

Figure 6 shows a [: S]schematic diagram of CTLA-4 VLD loop replacements. The constructs are labeled A-I. Construct A (CTLA-4 VLD: S2) represents the wild-type CTLA-4 extracellular V-domain, spanning residues 1-115. Constructs B (CTLA-4-Som1; PP2) and C (CTLA-4-Som1-Cys120; PP5) both contain the 14 residue somatostain polypeptide in CDR1. PP5 also carries a C-terminal extension containing Cys120. Construct D (CTLA-4-Som3; PP8) contains the 14 residue somatostatin polypeptide in place of CDR3. In construct E (CTLA-4-HA2:XX4), CDR2 has been replaced with a haemagglutinin tag. In construct F (CTLA-4-Som1-Som3: VV3), both CDR1 and CDR3 have been replaced with the somatostatin polypeptide. In

construct G (CTLA-4-Som-HA2-Som3: ZZ3) CDR1 and CDR3 are replaced with the somatostatin polypeptide whilst CDR2 is replaced with haemagglutinin tag. In construct H (CTLA-4-anti-lys:2V8), all three CDR loop structures have been replaced with the CDR loops from a camel anti-lysozyme V_HH molecule. Construct I (CTLA-4-anti-mel: 3E4) represents CTLA-4 VLD in which all three CDRs have been replaced by the VH CDR loops from anti-melanoma antibody V86 (Cai And Garen, 1997). PelB, cleavable pectate lyase secretion sequence (22 aa); flag, dual flag tag (AAADYKDDDDKAADYKDDDDK).

Figures 7A-I show[:] HPLC profiles of purified recombinant human CTLA-4 STMs. Recombinant CTLA-4 VLDs were expressed in E. coli host TG1 from vector pGC, purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12 HR column. The elution profiles of the purified proteins are shown. [Panel A,] 7A shows CTLA-4 DIMER (PP5); [Panel B,] 7B shows CTLA-4R (S2); [Panel C,] 7C shows CTLA-4-HA2 (XX4); [Panel D,] 7D shows CTLA-4-Som3 (PP8); [Panel E,] 7E shows CTLA-4-Som1 (PP2); [Panel F,] 7F shows CTLA-4-Som1-Som3 (VV3); [Panel G,] 7G shows CTLA-4-Som-HA2-Som3 (ZZ3); [Panel H,] 7H shows CTLA-4-anti-lys (2V8); [Panel I,] 7I shows CTLA-4-anti-mel (3E4).). Traces represent absorbance at 214 nm and are given in arbitrary units.

Figures 8A-E show a [: C]comparison by size exclusion FPLC analysis of affinity purified CTLA-4 constructs synthesised using bacterial expression vector pGC or pPOW. Recombinant human CTLA-4R or its loop variants were expressed in E. coli host TOP10F', purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12HR column. The elution profiles of proteins expressed from vector pGC are shown on the left, whilst proteins expressed from vector pPOW are shown on the right. [Panel A,] 8A shows wild-type CTLA-4 VLD (S2); [B,] 8B shows CTLA-4-Som1(PP2); [C,] 8C shows CTLA-4-Som3(PP8); [D,] 8D shows CTLA-4-Anti-lys(2V8); [E,] 8E shows CTLA-4-Som1-HA2-Som3(ZZ3).

Figures 9A-E show [: P]protein stability analysis. Stability of monomer preparations of CTLA-4 VLD and loop variant constructs was analysed by size exclusion fplc chromatography on a precalibrated superose 12 hr (Pharmacia) column following several cycles of freeze/thawing. Aliquots of each protein were tested immediately after peak purification and following two cycles of freeze/thawing. [A,] 9A shows CTLA-4 VLD (S2); [B,] 9B shows

CTLA-4-Som1 (PP2); [C,] 9C shows CTLA-4-Som3 (PP8); [D,] 9D shows CTLA-4-anti-lys (2V8); [E,] 9E shows CTLA-4-Som-HA2-Som3 (ZZ3).

Figures 10A-B show the [: L]lysozyme binding characteristics of CTLA-4-anti-lys construct 2V8. 10A shows ELISA analysis; 10B shows BIAcore analysis.

Figure 11 shows [: S]screening of CTLA-4 VLD phagemid library on immobilised Sh bleomycin.

Figures 12A-C show [: S]screening of CTLA-4 VLD libraries in solution. 12A shows an FD library (5 washes); 12B shows a phagemid library (2washes); and 12C shows a phagemid library (5 washes).--